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International application number: PCT/CA05/000409

International filing date: 18 March 2005 (18.03.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/553,955
Filing date: 18 March 2004 (18.03.2004)

Date of receipt at the International Bureau: 08 June 2005 (08.06.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



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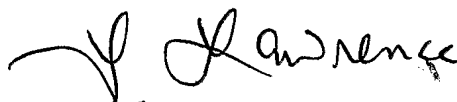
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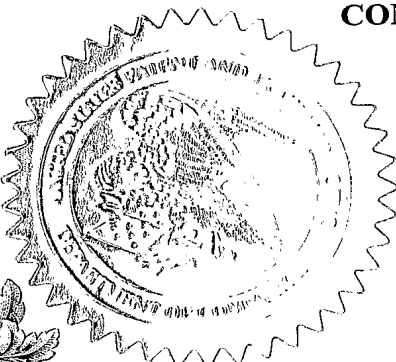
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FILING DATE: *March 18, 2004*

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16698 U.S. PTO

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Express Mail Label No.

19587 U.S. PTO
60/553955

031804

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Additional inventors are being named on the <u>1</u> separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
Endothelial protein C receptor (EPCR) haplotypes predict outcome of patients					
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Respectfully submitted,

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Enclosures:

1. Provisional application for patent cover sheet
2. Credit card payment for \$80.00 filing fee
3. Specifications, 20 pages
4. Drawings, 10 pages

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March 9, 2004

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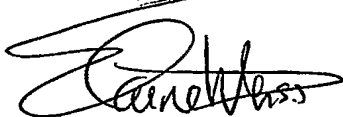
Re: Provisional Application for "Endothelial protein C receptor (EPCR) haplotypes
predict outcome of patients."

UBC file no: 04-097

Enclosed please find the necessary documents for filing a Provisional Patent Application for the above-identified technology on behalf of The University of British Columbia. Also enclosed is Credit Card payment form PTO-2038 to cover the cost of the \$80.00 application fee.

Thank you,

Sincerely,



Elaine A. Weiss, B.Sc, MBA
Technology Transfer Manager

Encl.



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PROVISIONAL APPLICATION COVER SHEET
Additional Page

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Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
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Number 2 of 2

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Title: Endothelial Protein C Receptor (EPCR) haplotypes predict outcome of patients.

Inventor: Keith Walley, Vancouver, CANADA
James Russell, Vancouver, CANADA

Abstract:

The invention involves characterization of polymorphisms in the Protein C and Endothelial protein C receptor (EPCR) genes that are associated with adverse outcomes in patients. Methodologies for screening haplotypes are described. EPCR and Protein C haplotype screening will be useful in identifying patients who would benefit from increased monitoring by healthcare professionals, and/or possible therapeutic intervention, when said patient become subject to inflammation due to systemic inflammation response syndrome (SIRS), bacterial infection, bacteraemia, sepsis, septic shock, organ dysfunction, and trauma.

Background of the Invention:

Septic and non-septic stimuli such as bacterial endotoxin and cardiopulmonary bypass (CPB), respectively, activate the coagulation system and trigger a systemic inflammatory response syndrome (SIRS). Recently, the pivotal PROWESS trial demonstrated that recombinant human activated protein C reduced mortality of patients who have severe sepsis or septic shock (4). Thus it is clear that protein C plays an important role in the systemic inflammatory response syndrome.

Interestingly, genotype has been shown to contribute substantially to outcome in inflammatory and infectious diseases (22, 23, 25, 28, 37, 38, 40). For example, Sorensen and colleagues (34) found that adopted children with a biologic parent dying of infectious diseases before age fifty had a relative risk of 5.8 of themselves dying of an infectious disease – a remarkably important genetic influence. This exceeded the genetic contribution to cancer risk (by five-fold) and even cardiovascular disease. Twin studies show, by a factor greater than two, greater concordance in monozygotic twins than dizygotic twins for tuberculosis, leprosy, poliomyelitis and hepatitis B (7). Since infection itself is not a genetic disease, this means that genetic variation in patients' inflammatory responses leads to significant differences in outcome. Thus it is reasonable to investigate the role of genotype when developing strategies to predict severity of individual patient inflammatory response. Because of the importance of protein C in SIRS, we examined the association/prediction of endothelial protein C receptor polymorphisms on outcome of critically ill patients who have SIRS.

We have previously found (PCT/CA03/00751) that the protein C -1641 A allele was associated with significantly lower survival, greater duration of SIRS, worse cardiovascular and respiratory dysfunction, and greater use of vasopressors in a critically ill population who had SIRS. These observations were confirmed in an analogous but completely independent SIRS population, i.e. the post CPBP patients in whom SIRS was induced by cardiac surgery and the cardiopulmonary bypass procedure without evidence of infection. Evidence for increased SIRS in this population was first, greater reduction in SRVI, second, greater vasopressor use at 1 hour post CPB, third impaired oxygenation, and fourth, increased pro- and anti-inflammatory cytokine expression as reflected by serum IL-6 and IL-10 concentrations respectively in patients who had protein C - 1641 A allele. The increased inflammatory cytokine expression of patients who had the protein C - 1641 A allele also provides evidence of biological plausibility in that these cytokines were chosen to be representative of an integrated inflammatory response (IL-6) and the counter regulatory anti-inflammatory response (IL-10).

The actions of APC are enhanced initially by binding to the endothelial cell protein C receptor (EPCR) and subsequently by binding to protein S. The protein C/EPCR interaction increases the rate of activation of protein C dramatically. The EPCR is primarily located on large vessels. The APC-protein S complex inactivates factors Va and VIIIa. Activated protein C also has pro-fibrinolytic action because APC inactivates plasminogen activator inhibitor-1 (PAI-1). Finally, APC inhibits LPS-induced proinflammatory cytokine production. NF κ B increases transcription of proinflammatory cytokines such as TNF α , IL1 β , IL-6, IL-8, and ICAM-1. APC inhibits LPS-induced nuclear translocation of nuclear NF κ B and TNF α in monocytes.

The EPCR gene is located on chromosome 20. A large number of polymorphisms have been observed in the EPCR gene (5, 9). The polymorphisms of EPCR are associated with decreased activity of the protein C/EPCR interaction and decreased generation of activated protein C (APC) (5). As a result, when protein C binds to EPCR there is less activation of protein C than would be expected to increase the risk of venous thromboembolic disease. Indeed, polymorphisms of EPCR have been associated with increased risk of venous thromboembolic disease in some (12, 41) but not all (10, 39) studies.

Using a novel haplotype-based analysis, the inventors have identified single nucleotide polymorphisms (SNPs) in the EPCR and protein C genes that identify a family of haplotypes (clade) that are associated with statistically significant differences in important measures of clinical outcome such as survival and organ dysfunction. The present invention describes a better strategy of predicting patients who are at a greater risk of an adverse outcome, thus enabling earlier intervention and facilitating patient-tailored therapy based on genotype.

Summary of the Invention:

5 The present invention is concerned with single nucleotide polymorphisms (SNPs), which form haplotypes within the endothelial protein C receptor (EPCR) gene, which are predictive of patient outcome should that patient experience inflammation. Examples of inflammation experienced by patients include, but are not limited to, systemic inflammation response syndrome (SIRS), acute lung injury/ acute respiratory distress syndrome (ARDS), bacterial infection, bacteraemia, sepsis, septic shock, organ dysfunction, and trauma. This invention is novel, as the respective grouping of haplotypes described in the invention predict risk of inflammation and sepsis and patient outcome much more accurately than previously identified EPCR polymorphisms.

15 In one aspect, the present invention provides the methodology required to screen patients in order to determine those at risk of an adverse outcome following inflammation. Genetic material is collected from the patient, most commonly by isolating leukocytes from the blood, but alternatively through a variety of biopsy methods, in order that the haplotype of the EPCR gene can be ascertained. Determination of the haplotype from the genetic material can be done through a variety of methods commonly described in the art, including, but not limited to sequencing, restriction fragment length polymorphism (RFLP) analysis, hybridization, oligonucleotide ligation assay, ligation rolling circle amplification, allele specific PCR, and single base-pair extension assays. Sequence data from any of the above mentioned assays could be stored in a database for future retrieval and haplotype analysis.

30 In another aspect of the invention, those patients at highest risk of inflammation are the infirm, elderly, and those individuals requiring hospitalization for a variety of reasons. These at risk individuals could be screened for the EPCR haplotypes associated with elevated EPCR and/or serum EPCR (sEPCR) such that those individuals can benefit from increased monitoring, and possible prophylactic treatments, in order to avoid the adverse effects of inflammation.

35 In another aspect of the invention, patients suffering from inflammation could be screened for the EPCR haplotypes associated with decreased EPCR and/or sEPCR such that those individuals can benefit from increased monitoring, and possible prophylactic treatments begun in order to avoid the adverse effects of inflammation.

40 In another aspect of the invention, the invention provides the methodology required to determine patient outcome following collection of genetic material and haplotype determination by analysing the EPCR gene, whereby the specific

EPCR SNPs that form the respective haplotypes are located in the sequence described in SEQ ID NO:1.

5 In another aspect, the invention further provides the methodology required to determine patient outcome following collection of genetic material and haplotype determination by analysing the EPCR gene, whereby 3 major haplotype clades could be defined by identifying the SNP at positions 6118 and 6196 of SEQ ID NO:1.

10 In another aspect, the invention further provides the methodology required to determine patient outcome following collection of genetic material by analyzing the EPCR gene for 6118A/6196G, 6118G/6196G or 6118A/6196C haplotypes, whereby those individuals display an adverse outcome. This outcome is due to decreased survival arising from inflammation due to organ dysfunction, SIRS,
15 sepsis, septic shock, bacterial infection, bacteraemia or trauma.

In another aspect, the invention further provides the methodology required to determine patient outcome following collection of genetic material by analysing the EPCR gene at position for the 6118A/6196C haplotype, whereby those
20 individuals do not display as severe an adverse outcome.

The sequence positions referred to in this invention and detailed in SEQ ID NO:1 refer to the sense strand of the EPCR gene. It will be obvious to a person skilled in the art that analysis could be conducted on the anti-sense strand to
25 determine patient outcome.

The invention further provides for kits useful in carrying out the methods of the invention.

30

Brief Description of the Drawings:

35

Figure 1. Haplotype structure of the EPCR gene in Caucasians. EPCR haplotypes, inferred using PHASE from available data. Each row represents a polymorphic site within the EPCR gene and is labelled on the left with the position in the gene. Each column represents one of
40 the inferred haplotypes. MEGA II was used to sort haplotypes into clades separated by heavier lines. Haplotypes within each clade are very similar while clades differ substantially from each other.

Figure 2. EPCR haplotype clades. An unrooted phylogenetic tree, of haplotypes from Figure 1, was drawn using MEGA II where line length reflects relative evolutionary distance.

5 Figure 3. Days alive and free of acute lung injury/ARDS by EPCR haplotype clade.

Figure 4. Sequence context of EPCR SNPs
<http://pga.gs.washington.edu/data/procr/procr.snpcontext.fasta>

10

Detailed Description of the Invention:

Definitions

- 5 Allele — One of the variant forms of a gene at a particular locus, or location, on a chromosome. Different alleles produce variation in inherited characteristics such as hair color or blood type. In an individual, one form of the allele (the dominant or major one) may be expressed more than another form (the recessive or minor one).
- 10 Clade — A group of haplotypes that are closely related phylogenetically. For example, if haplotypes are displayed on a phylogentic (evolutionary) tree a clade includes all haplotypes contained within the same branch.
- 15 Genetic Material — Genetic material refers to nucleic acids, whether deoxyribonucleic acid or ribonucleic acid, isolated from cells acquired from tissue or organisms.
- Genotype — Genotype refers to the genetic makeup of an organism.
- 20 Haplotype — The set of genes, comprised of one allele of each gene, which make up the genotype.
- Phenotype — Phenotype refers to the observable characteristics of an organism produced by the organism's genotype interacting with the environment.
- 25 Single Nucleotide Polymorphism (SNP) — A SNP is a place in the genetic code where DNA differs from one person to the next by a single nucleotide base pair. These slight genetic variations between human beings may predispose some people to disease and explain why some respond better to certain drugs.
- 30

Methods

- 35 Patient Cohort — All patients admitted to the Intensive Care Unit (ICU) of St. Paul's Hospital were screened for inclusion. This ICU is a mixed medical – surgical ICU in a tertiary care, university-affiliated teaching hospital of the University of British Columbia. SIRS was considered present and the patients included in the study when patients met at least two of four SIRS criteria. The SIRS criteria were 1) fever ($>38^{\circ}\text{C}$) or hypothermia ($<35.5^{\circ}\text{C}$), 2) tachycardia
- 40 (>100 beats/min in the absence of beta blockers, 3) tachypnea (>20 breaths/min) or need for mechanical ventilation, and 4) leukocytosis (total leukocyte count $>11,000/\mu\text{L}$). Patients were included in this cohort on the calendar day on which the SIRS criteria were met. To decrease the confounding

influence of population admixture secondary to ethnic diversity on associations between genotype and phenotype, only Caucasian patients were studied.

- 5 700 consecutive critically ill patients admitted to St. Paul's Hospital ICU were screened for inclusion into our study. Of these, 600 patients (94%) met the inclusion criteria of having at least two out of four SIRS criteria. From this group, 222 patients were Caucasian and were successfully genotyped and used as our final cohort for analysis.
- 10 Clinical Phenotype — Our primary outcome variable was 28 day mortality. Secondary outcome variables were measures of organ dysfunction and of the intensity of SIRS and sepsis.
- 15 Baseline demographics that were recorded included age, gender, medical or surgical diagnosis for admission (according to APACHE III diagnostic codes, and admission APACHE II score(17-19). After meeting the inclusion criteria, data were recorded for each 24 hour period (8 am to 8 am) for 28 days to evaluate organ dysfunction, SIRS, sepsis, and septic shock.
- 20 Measures of organ dysfunction – Organ dysfunction for each organ system was defined as being present during a 24-hour period if there was evidence of at least moderate organ dysfunction using the Brussels criteria (Table 1) (33). Because data were not always available during each 24 hour period for each organ dysfunction variable, we used the "carry forward" assumption as defined
- 25 previously (1). Briefly, for any 24 hour period in which there was no measurement of a variable, we carried forward the "present" or "absent" criteria from the previous 24 hour period. If any variable was never measured, it was assumed to be normal.
- 30 To further evaluate cardiovascular, respiratory, and renal function we also recorded, during each 24 hour period, vasopressor support, mechanical ventilation, and renal support, respectively. Vasopressor use was defined as dopamine > 5 µg/kg/min or any dose of norepinephrine, epinephrine, vasopressin, or phenylephrine. Mechanical ventilation was defined as need for
- 35 intubation and positive airway pressure (i.e. T- piece and mask ventilation were not considered ventilation). Renal support was defined as hemodialysis, peritoneal dialysis, or any continuous renal support mode (e.g. continuous veno-venous hemodialysis). In addition, the severity of respiratory dysfunction was assessed by measuring the occurrence of acute lung injury at the time of
- 40 meeting the inclusion criteria. Acute lung injury was defined as having a PaO₂/FiO₂ ratio <300, diffuse infiltrates pattern on chest radiograph, and a CVP <18 mm Hg.

Measures of the intensity of SIRS and sepsis – Each of the four SIRS criteria were recorded as present or absent during each 24-hour period. Sepsis was defined as the presence of two or more SIRS criteria plus the presence of a known or suspected infection during the 24-hour period. Cultures that were judged to be positive due to contamination or colonization were excluded. Septic shock was defined as the presence of sepsis plus significant hypotension (systolic blood pressure <90 mm Hg or the need for vasopressors) during the same 24-hour period.

Days alive and free - To assess duration of organ dysfunction and to correct organ dysfunction scoring for deaths in the 28 day observation period, we calculated days alive and free of organ dysfunction (DAF) as previously reported (3). Briefly, during each 24-hour period for each variable, DAF was scored as 1 if the patient was alive and free of organ dysfunction (normal or mild organ dysfunction). DAF was scored as 0 if the patient had organ dysfunction (moderate, severe, or extreme) or was not alive during that 24-hour period. Each of the 28 days after meeting the inclusion criteria was scored in each patient in this fashion. Thus, the lowest score possible for each variable was zero and the highest score possible was 28. A low score is indicative of more organ dysfunction as there would be fewer days alive and free of organ dysfunction.

Microbiology – Microbiological cultures were taken for any patients who were suspected of having an infection. As this is a cohort of critically ill patients with SIRS, most patients had cultures taken. Positive cultures that were suspected of having been contaminated or colonized were excluded. Positive cultures that were deemed to clinically be clinically irrelevant were also excluded. Cultures were categorized as gram positive, gram negative, fungal or other. The sources of the cultures were respiratory, gastrointestinal, skin, soft tissues or wounds, genitourinary, or endovascular.

Haplotypes and Selection of htSNPs — Using unphased Caucasian genotypic data from the Coriell registry (from pga.mbt.washington.edu) (32), we inferred haplotypes of EPCR gene using PHASE software (36). We then used MEGA 2 to infer a phylogenetic tree to identify major haplotype clades (20). Haplotypes were sorted into clades according to this phylogenetic tree and this haplotype structure was inspected to choose "haplotype tag" SNPs (htSNPs) (11, 14). We chose 3 ht SNPs that identified 5 major haplotype clades of EPCR in Caucasians.

Blood collection and processing – The buffy coat was extracted from whole blood and samples transferred into 1.5 ml cryotubes and stored at -80°F. DNA was extracted from the buffy coat using the Qiagen DNA Blood Mini Kit. The genotypic analysis was performed in a blinded fashion, without clinical information.

Genotyping — The identified htSNPs were genotyped using a MALDI-TOF approach (6). Briefly, PCR was used to amplify the region surrounding the variant sites. Each PCR reaction was then subjected to a 'mini-sequencing reaction' resulting in a population of extended and unextended oligonucleotides for each reaction that could be distinguished from each other by mass. Prior to mass determination, each reaction was purified by reverse-phase chromatography and spotted on the MALDI plates. Linear delayed mass spectrometry was used to assess the masses of each reaction. Genotypes are resolved using suitable allele-calling software.

Statistical Analysis — A cohort study design was used. Rates of dichotomous outcomes (28-day mortality, sepsis and shock at onset of SIRS) were compared between haplotype clades using a chi-squared test, assuming a dominant model of inheritance. Differences in continuous outcome variables between haplotype clades were tested using ANOVA. 28-day mortality was further compared between haplotype clades while adjusting for other confounders (age, sex, and medical vs. surgical diagnosis) using a Cox regression model, together with Kaplan-Meier analysis. Haplotype clade relative risk was calculated. This analysis was performed in the entire cohort, and subsequently in sub-groups of patients who had sepsis at onset of SIRS, and patients who had septic shock at onset of SIRS. Genotype distributions were tested for Hardy-Weinberg equilibrium (13). We report the mean and 95% confidence intervals. Statistical significance was set at $p < 0.05$. The data was analyzed using SPSS 11.5 for Windows and SigmaStat 3.0 software (SPSS Inc, Chicago, IL, 2003).

Discussion

Our key finding was that haplotype clades 2 and 3 of EPCR were associated with significantly more acute lung injury/ARDS as reflected by fewer days alive and free of lung injury/ARDS in a cohort of 222 critically ill Caucasian patients who had SIRS. We subsequently found that the 6196 G/G genotype was individually associated with fewer days alive and free of acute lung injury. The 6196 G allele is contained within both haplotype clade 2 and clade 3.

EPCR receptors are located primarily on the large arterial and venous vessels. Protein C binding to the EPCR increases the activation of protein C by several thousand fold (REF). The EPCR haplotypes appear to decrease the conversion of protein c to activated protein C and increase the risk of local coagulation in response to a procoagulant stimulus.

Polymorphisms of the endothelial protein C receptor gene are associated with variable risk of deep venous thrombosis (31). To our knowledge, this is the first

report of EPCR polymorphisms in critically ill patients. Furthermore, this is the first study to show that patients who had the EPCR haplotype clades 2 and 3 had more acute lung injury/ARDS than did haplotype clade 1. Other studies of polymorphisms of XX have been associated with altered risk of acute lung injury/ARDS in the critically ill. ACE etc.

Coagulation is important in the pathogenesis of acute lung injury/ARDS (30). Our study suggests that the protein C/EPCR pathway may be important in the genesis of acute lung injury because the EPCR haplotype 2 and 3 were associated with more acute lung injury. We speculate that impaired balance of the coagulant vs. anticoagulant forces in the lung and/or in the systemic circulation could account for the increased risk of acute lung injury/ARDS associated with the endothelial protein C receptor haplotype 1. Early in ALI, fibrin is deposited in the alveoli. The deposition of fibrin is caused by a relative increase of procoagulant vs. anticoagulant/fibrinolytic activity. Fibrin in the alveoli increases the inflammatory response by increasing chemotaxis and by increasing permeability. Previous studies have shown that procoagulant activity is increased and fibrinolytic activity is decreased in the BALF of patients who have ARDS. More specifically, tissue factor associated VII (procoagulant) and PAI-1 (anti-fibrinolytic) activity was increased. Furthermore, patients who have multiple trauma who develop ARDS have higher levels of procoagulant activity than patients who have trauma but who do not get ARDS do.

Recent evidence suggests there is "cross-talk" amplification of the coagulation and inflammatory cascades by each other (24). TNF- α and IL-6 both increased tissue factor expression by increasing PAI-1, thereby activating the extrinsic pathway of coagulation (29). Furthermore, pro-inflammatory cytokines such as TNF- α and IL-1 also enhance coagulation by decreasing thrombomodulin (27) and inhibiting fibrinolysis. A systemic inflammatory response decreases circulating levels and action of APC by decreasing levels of protein S (co-factor), by decreasing thrombomodulin expression on endothelial cells, by increasing cleavage of endothelial cell thrombomodulin (21, 26), by down-regulating EPCR expression on endothelial cells, and by protein C consumption (8). These mechanisms could be altered by the genotype of the EPCR to increase or decrease the coagulation/anticoagulation balance.

Conversely, intravascular coagulation induces an inflammatory response. Coagulation of blood in vitro increases production of IL-8 (15, 16) and endotoxin synergistically increases the production of IL-8 by monocytes. Also, factor Xa, thrombin and fibrin increase synthesis of IL-6, IL-8, and MCP-1 (35). Thus, the inflammatory cascades of SIRS accentuate the prothrombotic imbalance leading to DIC. Thus, inflammation leads to excessive coagulation - which further amplifies inflammation.

Sepsis alters the interaction of protein C with EPCR by several mechanisms. First, endotoxin and cytokines, such as TNF- α , down-regulate EPCR expression on endothelial cells, thereby decreasing action of APC. There are other mechanisms of protein C alteration in sepsis. Second, acute inflammatory states decrease levels of the free form of protein S that decreases APC function because free protein S is an important co-factor for APC. Third, sepsis, acute inflammation and cytokines decrease thrombomodulin expression on endothelial cells. Septic shock increases circulating levels of thrombomodulin, a sign of increased cleavage of endothelial cell thrombomodulin. Finally, severe septic states such as meningococemia result in protein C consumption. Indeed, depressed protein C levels correlate with purpura, digital infarction and death in meningococemia. Lower protein C levels in sepsis may be important in patients who have EPCR haplotype 1 and could alter the risk of acute lung injury.

There are several strengths of this association study of polymorphisms and haplotypes of EPCR. First, this is the first report of the associations between EPCR with acute lung injury in the critically ill. Second, we studied a cohort of Caucasian patients to minimize the risk of false positive spurious associations of genotype with phenotype due to population stratification. Finally, we used days alive and free of acute lung injury as a sensitive clinical phenotype because that has been sensitive to differences between groups in other studies and in RCT's of new therapy (1, 2).

In summary, haplotype clades 2 and 3 of EPCR were associated with more acute lung injury in the critically ill Caucasian adults. Therefore, the EPCR haplotype could have diagnostic and prognostic use in the critically ill. It is unknown whether the effects of rhAPC (Xigris) on acute lung injury in patients who have SIRS, sepsis, or septic shock differs in patients who have the EPCR haplotype 1 compared to haplotypes 2 and 3.

Examples:

252 consecutive critically ill patients admitted to the ICU of St. Paul's Hospital were screened for inclusion. Of these, 223 Caucasian patients were successfully genotyped and make up the cohort of this study.

Example 1

Haplotype clade deduction

Using PHASE software (36), we were able to infer 7 haplotypes of the EPCR gene from complete sequencing of EPCR in 23 Caucasians from the Coriell Cell Repository (from pga.mbt.washington.edu) (32) (Figure 1). We used phylogenetic analysis to sort these haplotypes into 3 clades (Figure 2), and

selected the htSNPs A6118G (rs867186) and G6196C (rs9574) to uniquely identify each haplotype clade (Figure 1). 222 Caucasian patients admitted to our ICU with SIRS and successfully genotyped for the A6118G and G6196C polymorphisms were included in this study. The genotype frequencies of A6118G and G6196C are shown in Table 2. These alleles were in Hardy Weinberg equilibrium in our population. Haplotype clade 1, defined by 6118A/6196C, occurred with a frequency of 37%. Haplotype 2, defined by 6118A/6196G, occurred in 39% of our cohort, while haplotype 3, defined by 6118G/6196G, occurred in 24% of our cohort.

Table 3 shows that there were no significant differences in baseline characteristics of associated with haplotype clades 1, 2, or 3. Patients were of similar age had similar APACHE II scores. There was a trend to more males in haplotype 3 (Table 3). There was no difference in the frequency of sepsis or septic shock at the time of onset of SIRS (Table 3).

Our key finding was that the EPCR haplotype clades 2 and 3 were associated with fewer days alive and free of acute lung injury /ARDS injury than haplotype clade 1 (Figure 3) in our entire cohort of patients with SIRS. There was also a trend ($p < 0.07$) to more acute renal dysfunction (expressed as fewer days alive and free of acute renal dysfunction) in haplotype clades 2 and 3. These associations were not seen in sub-groups of patients with sepsis at onset of SIRS, or those patients with septic shock at onset of SIRS.

Example 2

Haplotype patient outcome

There was no difference between between haplotype clades 1, 2 or 3 in 28 day mortality (Table 3). There were no associations of EPCR haplotypes with cardiovascular, neurologic, hepatic or coagulation dysfunction (Table 4). There was also no association of haplotype or genotype with days alive and free of ventilatory, vasopressor or renal support (Table 5).

When examined individually, we found that neither htSNP was associated with a difference in baseline characteristics (age, sex, medical vs. surgical diagnosis, APACHE II score), 28-day mortality, or days alive and free of organ dysfunction, with the exception of acute lung injury. The EPCR 6196 G/G genotype was associated with significantly fewer days alive and free of acute lung injury/ARDS than the 6196G/C and C/C genotypes combined (16 days vs. 20 days, $p < 0.006$), again indicating more acute lung injury/ARDS. The 6196 G allele is contained within both haplotype clades 2 and 3.

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TABLE 1
Brussels Organ Dysfunction Scoring System

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ORGANS	Free of Organ Dysfunction		Clinically Significant Organ Dysfunction		
	Normal	Mild	Moderate	Severe	Extreme
<u>Cardiovascular</u> Systolic BP (mmHg)	>90	≤90 Responsive to fluid	≤90 Unresponsive to fluid	≤90 plus pH ≤7.3	≤90 plus pH ≤7.2
<u>Pulmonary</u> P _a O ₂ /F _i O ₂ (mmHg)	>400	400-301	300-201 Acute lung injury	200-101 ARDS	≤100 Severe ARDS
<u>Renal</u> Creatinine (mg/dL)	<1.5	1.5-1.9	2.0-3.4	3.5-4.9	≥5.0
<u>Hepatic</u> Bilirubin (mg/dL)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	≥12
<u>Hematologic</u> Platelets (x10 ⁵ /mm ³)	>120	120-81	80-51	50-21	≤20
<u>Neurologic</u> (Glasgow Score)	15	14-13	12-10	9-6	≤5
Round Table Conference on Clinical Trials for the Treatment of Sepsis Brussels, March 12-14, 1994 (33)					

TABLE 2
Genotype frequencies of EPCR haplotype tag SNPs A6118G and C6196G

	Genotype Frequencies			Allele Frequencies		p*
	AA	AG	GG	A	G	
A6118G	81%	19%	0%	90.5%	9.5%	0.994
G6196C	CC	CG	GG	C	G	0.986
	23%	41%	36%	44%	56%	

* exact test of Guo and Thompson to test for Hardy-Weinberg equilibrium (13)

TABLE 3

Baseline characteristics and mortality of 222 critically ill patients who had SIRS

Haplotype Clade	Mean Age	Gender (% Male)	Diagnosis for admission (% Surgical)	Mean APACHE II	Sepsis on Admission	Septic Shock On Admission	28-day Mortality
1	61	63%	30%	20	54%	43%	31%
2	59	65%	31%	19	61%	50%	37%
3	63	79%	33%	20	60%	52%	33%
p	NS	0.06	NS	NS	NS	NS	NS

TABLE 4

Days alive and free of (DAF) SIRS and Key Organ Dysfunction in 222 critically ill patients who had SIRS

Haplotype	DAF SIRS 4/4	DAF SIRS 3/4	DAF ALI	DAF CNS	DAF CVS	DAF COAG	DAF RENAL	DAF HEPATIC
1	22.	22	20	21	21	24	19	20
2	20	20	17	19	19	24	17	19
3	21	21	18	20	20	25	18	20
p	NS	NS	0.006	NS	NS	NS	0.07	NS

5

TABLE 5

Days alive and free of (DAF) Life Support in 222 critically ill patients who had
SIRS

Haplotype	DAF Vasopressors	DAF Renal Support	DAF Ventilatory Support
1	19	19	15
2	18	18	14
3	19	19	15
p	NS	NS	NS

5

Figure 1. Haplotypes and haplotype clades of the endothelial protein C receptor gene.

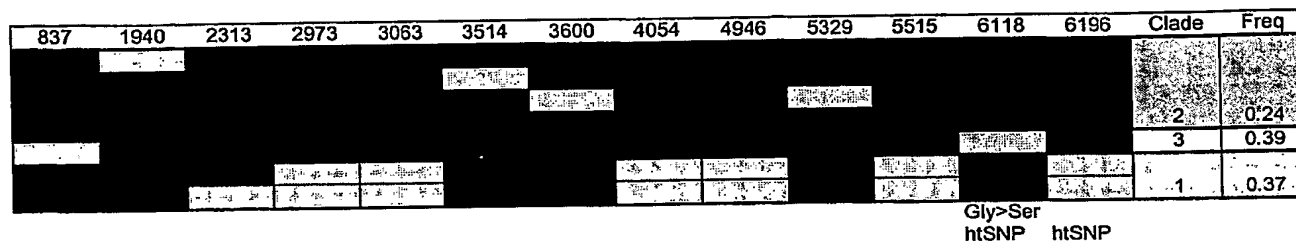
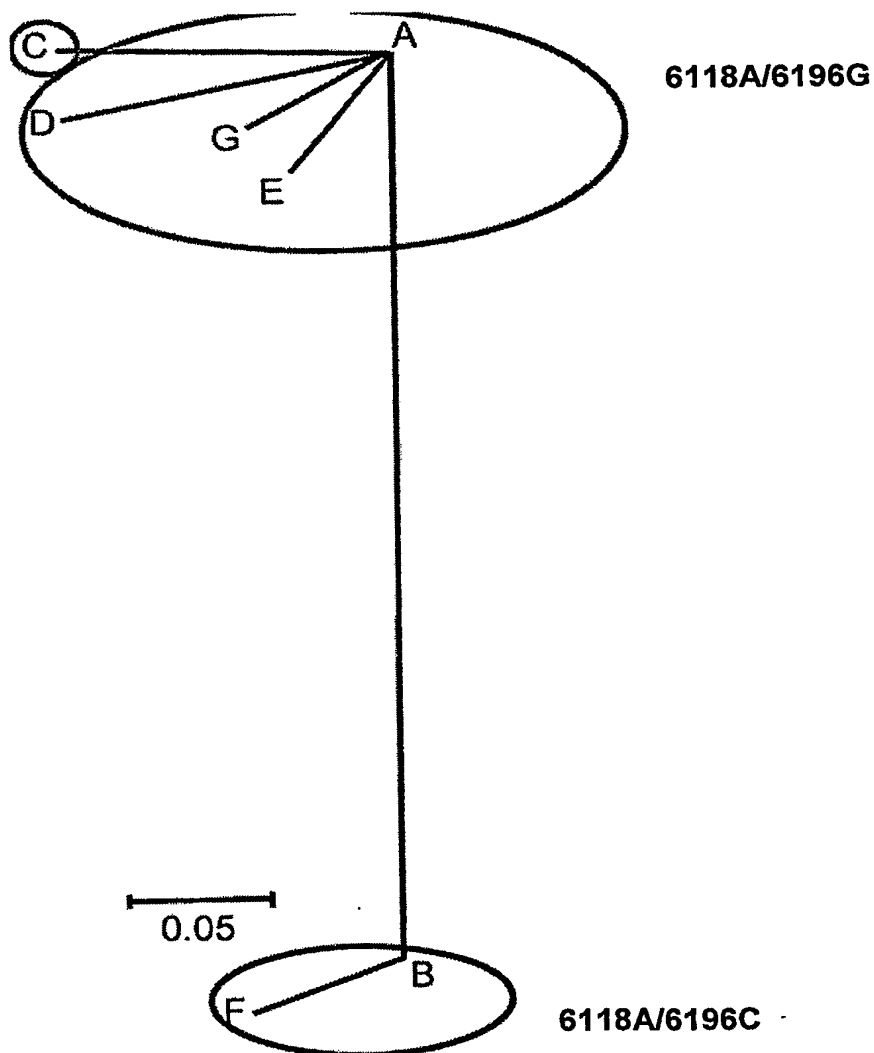


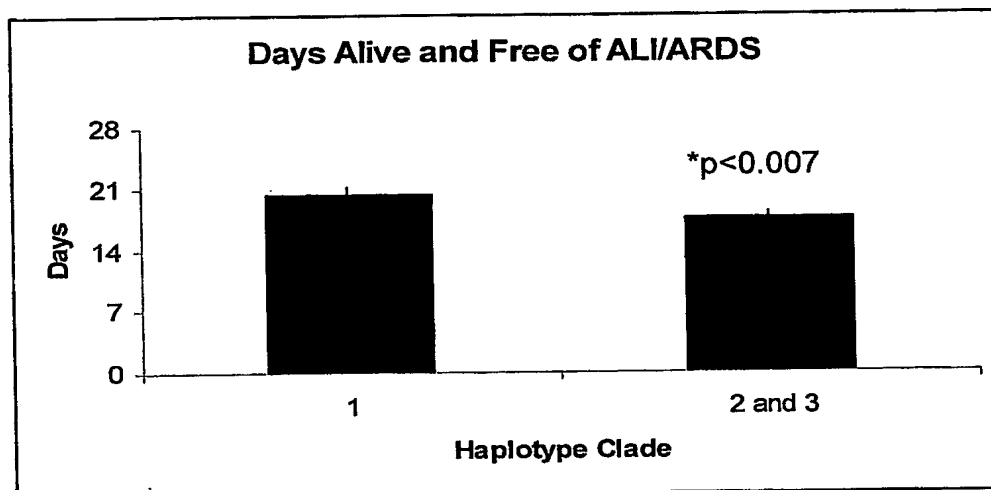
Figure 2. Phylogenetic tree of EPCR haplotypes generated with MEGA2 software.

6118G/6196G



5

FIGURE 3. Days Alive and Free of Acute Lung Injury/ARDS by EPCR Haplotype Clade



5

FIGURE 4. Sequence context of EPCR SNPs

<http://pga.gs.washington.edu/data/procr/procr.snpcontext.fasta>

```
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  TGTCCACTAATAAATTATGACCTCAGTTTCAAAAAGATTGCTTTAGGTAA
  S
  CAATCATCTTCTGAGATTTATACAGATTGCTCATAATTCTCTCCTATTTT
  >PROCR-000951
10 GCAGTGAAGTCTTTTACACTCATTTTATGACTACTTCTGAGACCAAGATC
  Y
  CGGATTATGTAATTGTTATTTACTTAAAATTCTGGTAAAATGTAGCCATT
  >PROCR-001940
  AGAAAGGGGAAGTGAAGGTGATGGTAGATAGGGGTACATCTAGGGGAGAC
  R
15 GGAAGAGGCTCAGAAGAGAAGAGAAATGGAGGGAATGGGAAGACCCCTGGG
  >PROCR-002313
  CCAGTATTCAATGAGTGCTCACTATGGTTAATACATGTATTGACCCATTT
  M
  ACTTGACAAAACCCCTAAAGGTGGGTAATATTATTACTATCTCCATTTTA
20 >PROCR-002674
  TGTGTGTTTGGAGACAGCCAGGTAGTATCCCGTGAGATACACACTAATAT
  R
  TGGTGGTCTGGGATCACTGAAACAGACACACTGTGTCTCGTGGGGCATCA
  >PROCR-002973
25 GAGTAGCTGGGACTACAGGCATGTACCACCACGCCTGGCTAATATTTGTA
  Y
  TTTTAGTACAGATGGGGTTTCGCCATGTTGGCCAGGCTGGTCTTGAATCC
  >PROCR-003063
  GTCTTGAATCCCTGACCTCAAGTGATCCGCCCCGCTCGGCCTCCCAAAGT
  R
30 CTGGGATTACAGGCATGAGCCACCGCGCCAGTCTCTGAGCTGGGTCTTA
  >PROCR-003402
  CTGCCTACGTGCAAACCTTGGCTCTGCTACACTATCTCTGTCTCAGTTTC
  S
35 CATGTAGACTGGGGTTAATAATAGTAGCTATTGCATTAAGCCACTGGGGA
  >PROCR-003514
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  R
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  Y
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  >PROCR-003615
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  >PROCR-004054
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50 Y
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55 CAATTTCTTTTCTCAAAGCACCAAGCACCCTCCGTCCCCCTTCCCCCAC
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10 Y
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15 GTTTCATCATTGCTGGTGTGGCTGTAGGCATCTTCCTGTGCACAGGTGGA
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SEQ ID. NO. 1

Human endothelial protein C receptor (EPCR) 7353 bases

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SEQ ID. NO. 2

>gi|16950557|gb|AF106202.2| Homo sapiens endothelial cell protein C receptor precursor (EPCR) gene, promoter region and complete cds
8167 bases

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